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THE NATURE OF THE EMBRYO SAC OF PEPPEROMIA¹

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(WITH PLATES XXXI-XXXIII)

At the suggestion of Professor DUNCAN S. JOHNSON, I undertook the cytological study of the development of the embryo sac of several species of Peperomia, with the purpose of finding out whether the development of this genus offered any support to the idea, recently advanced by several investigators, that when a row of megasporangia is not formed, each of the first four nuclei of the embryo sac is to be regarded as a megasporangium nucleus. The results found were exceptional and may be of interest, as they seem to throw some light on this question and also on the nature of the embryo sac of Peperomia.

For the investigation, Professor JOHNSON turned over to me material of three species which he had collected for this purpose. Material of *P. arifolia* was collected in the greenhouses in Baltimore. The material was fixed in chrom-acetic or Fleming's solutions. The sections were cut 10μ thick and stained with Fleming's triple or Haidenhain's iron-alum hematoxylin. The latter stain was used alone or counterstained with gentian-violet or eosin.

This paper does not pretend to be a study of the whole life-history of Peperomia, but deals in detail only with the development of the embryo sac. It is hoped, however, that this will throw some light on the origin of the peculiarities which have been described in its later development.

The sixteen-nucleate embryo sac of Peperomia was discovered in *Peperomia pellucida* by CAMPBELL ('99), who, however, misinterpreted some of its features. JOHNSON ('00) describes the mature sac of *P. pellucida* as containing one egg, one cell with the position of a synergid, six nuclei which are cut off singly against the wall of the sac and finally degenerate, and eight which fuse to form the endosperm nucleus. The archesporium consists of a single hypodermal cell which cuts off a single parietal cell and then forms the embryo sac. In a later

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paper CAMPBELL ('01) agrees with this description, except that he does not think that there is always in the mature sac a single cell which has the position of a synergid.

That chromosome reduction takes place in the first division of the embryo sac nucleus was indicated by the presence of synapsis before this division in *P. hispidula*, as reported by JOHNSON before the Botanical Society of America at New Orleans in 1905.

CAMPBELL ('01) thinks that the embryo sac of Peperomia is a primitive one, while JOHNSON ('00) considers its peculiar structure as derived. In this paper it will be shown that the embryo sac is made up of the descendants of four nuclei which are apparently the nuclei of four megasporangia, and that these nuclei have, by the loss of dividing walls, come to lie in the same cell. Some of the peculiar features of the mature sac are probably connected with this fact.

For the sake of convenience, each of the four species will be described separately, after which the general considerations will be discussed.

Peperomia Sintensis

The material of this species was collected in Jamaica by Mr. W. R. MAXON and identified by M. CASIMIR DE CANDOLLE.

The development of the flower and of the mother cell in the nucellus agrees with that described for *P. pellucida* by JOHNSON ('00). The flower consists of two stamens and a carpel in the axil of the bract. The ovule (*fig. 28*) is single and orthotropous, with a single integument (*fig. 28, i*), which makes its appearance about the time that the tapetum is cut off from the archesporium (*fig. 2*).

The archesporium arises in the apex of the nucellus as a single hypodermal cell (*fig. 1*), which is clearly distinguished from the surrounding cells by its larger size and more densely staining contents. At the micropylar end this cuts off a single parietal cell (*fig. 2*), and then, without giving rise to any other cells, forms the embryo sac. The parietal cell divides first by an anticlinal wall and then by repeated divisions gives rise to a mass of tissue between the embryo sac and micropyle (*figs. 4, 28, t*).

Owing to the scarcity of young material, I was unable to count the chromosomes in the division cutting off the tapetum, but in the tapetum and in the nucellus there were regularly about sixteen chro-

mosomes. This is double the number found in the embryo sac, as will be described later. The chromosomes are small and short, and are therefore readily counted in a cross-section of a spindle at metaphase. *Fig. 2* shows such a section in the tapetum, while *fig. 3* represents a longitudinal view of a vegetative nucleus at a slightly older stage. Up to this time there certainly seems to have been no chromosome reduction, and nothing resembling megasporogenesis.

The single sporogenous cell (*fig. 2*) which is left after the cutting-off of the tapetum and which is to form the embryo sac is apparently a megasporangium mother cell, as will be shown below. Its nucleus divides to two, four, eight, and finally in the mature sac to sixteen nuclei.

The first division is heterotypic and takes place as follows. The resting nucleus (*fig. 4*) shows a meshwork of linin along which chromatin granules are scattered. In the center of this meshwork is a large clear space containing a large nucleolus. After considerable growth the nucleus goes into synapsis. The meshwork contracts rapidly around, or to one side of, or even at some distance from, the nucleolus, into a mass in which very little detail can be made out. *Fig. 5* represents an early stage of synapsis, while *fig. 6* is probably older. No evidence of a fusion of spirems was seen either before or during the early stages of synapsis.

At the end of synapsis the mass loosens up, and later appears in the form of a spirem, along which single granules are scattered at rather regular intervals (*fig. 7*). The spirem is apparently continuous and becomes loosely coiled, and the granules divide along the longitudinal axis of the spirem. A small portion of such a stage is shown in *fig. 8*. After this the spirem divides longitudinally and the two halves may diverge considerably in places (*fig. 9*), but later they come together again and all apparent traces of the division are lost. While this is taking place, the spirem is beginning to be arranged in loops (*fig. 10*), and is still apparently continuous, the loops being rounded at the ends. It does not seem possible that this appearance can have anything to do with the splitting just described. The looping becomes more pronounced and the spirem segments transversely in such a way that the loops give rise to chromosomes. There are eight of these, the haploid number, and they are apparently formed by the

coming together side by side of parts of the spirem that before were arranged end to end. The chromosomes then contract and show the twisted appearance characteristic of the heterotypic division (*fig. 11*).

After considerable contraction they have the appearance of two irregular rounded masses lying together. Sometimes these are seen to be connected by strands (*fig. 13*), and just before this, when the constituent halves are about twice as long as wide, they are sometimes placed end to end with a constriction between them (*fig. 12*).

It would seem from this that the two halves originally placed end to end in the spirem, then side by side in the loops, again come to lie end to end, and that each half probably represents a chromosome (cf. *fig. 16*). The strands connecting the two halves are often seen to be double (*figs. 12, 13*), and they might always be so if seen in the right plane. Besides this, the halves sometimes show evidence of being double. This may be due, as is often supposed, to the split previously described. Before the spindle is formed the two halves come together, producing somewhat elongated chromosomes (*fig. 14*). About this time the nucleolus begins to fragment and to be thrown out into the cytoplasm. After the spindle is formed (*fig. 15*) the chromosomes divide transversely to their long axes (*fig. 16*). From what has been said it seems evident that this division separates parts of the spirem which were originally placed end to end and that it is therefore a transverse division. This is then the heterotypic and reducing division.

As the chromosomes approach the poles they become crowded together (*fig. 17*) and surrounded by a clear space. While this is going on, the chromosomes lose their distinct outlines and a nucleolus makes its appearance in their midst. While the chromosomes lose their distinct outline and probably also some of their substance, they seem nevertheless to be represented by irregular masses during most if not all of the period between the first and second divisions (*figs. 18–20*). Between the succeeding divisions they seem to go to pieces to a much greater extent (cf. *figs. 22–25*). Before the formation of the spindle, the chromosomes appear as double structures, consisting of two rods lying side by side. It may be that these represent the two halves of the spirem seen in the prophases of the first division.

and that the second division completes the longitudinal separation begun in the first.

The first two divisions differ from those in *P. pellucida* in that there are formed evanescent walls separating the daughter nuclei. When the daughter nuclei of the first division have begun to be organized, an equatorial plate is formed on the spindle (fig. 17). This grows until it becomes a wall stretching across the embryo sac (fig. 18). The plane of this wall is not constant, but it may extend longitudinally or transversely across the sac, or take any intermediate position, and may also separate the sac into equal or unequal parts (figs. 18–20). It persists only for a short time, disappearing before the next division or remaining as a remnant after it (fig. 21). There is no trace of it later in the four-nucleate stage. When the two nuclei divide to four, plates are formed on both spindles. One of these never becomes very prominent, but the other forms a wall separating one nucleus from the other three (fig. 22). The position of this wall is variable, as was the one in the two-nucleate stage. It may cut off a nucleus at either end or any side of the sac, but generally it appears at the lower end. This wall, like the first, persists for only a short time. It generally disappears before the next division (fig. 23), but may persist as a remnant after it. As in *P. pellucida*, the four nuclei assume the position of the nuclei of a tetrad of microspores. The walls just described are apparently megasporangium walls. This, however, will be discussed later.

In the next two divisions all of the nuclei divide simultaneously, giving eight, and then in the mature sac sixteen nuclei. The nuclei of the eight-nucleate stage and of the mature sac are arranged about the periphery of the sac (figs. 24–28). As in *P. pellucida* (JOHNSON '00), one of the sixteen nuclei becomes an egg, another has the position of a synergid, six are cut off singly against the wall and finally degenerate, while eight fuse to form the endosperm nucleus. Fig. 29 shows a sac in which fertilization is taking place. Four of the peripheral nuclei are shown, while five others are fusing to form the endosperm nucleus. One of these latter was probably formed by the fusion of two. The other two fusing nuclei, as well as two peripherals, are in another section.

In the division of the four nuclei to eight no cell plates are formed.

Fig. 24 represents a stage in which they should be apparent if present. In the last division (*fig. 26*), cell plates are formed on all the spindles. These give rise to walls cutting off all of the nuclei except those which are to fuse to form the endosperm nucleus, but leaving these eight free in the cytoplasm (*figs. 27, 28*). These walls are thin at first and might be easily overlooked, but later they become much more prominent. In the eight-nucleate stage two nuclei are always found together at the micropylar end (*fig. 25*). The presence of spindles show these to be sisters (*fig. 24*). In the last division one of these gives rise to the egg, the other to the nucleus with the position of a synergid (*figs. 26-29*). The sisters of the egg and synergid fuse with the sisters of the six peripherals to form the endosperm nucleus. As in *P. pellucida* (JOHNSON '00) the peripherals are arranged singly against the embryo sac wall (*fig. 29*) and finally degenerate. About the time of fertilization the eight nuclei which form the endosperm nucleus migrate toward the base of the sac and fuse into one large nucleus.

STRASBURGER ('05) assumes that owing to their position the polar nuclei in the ordinary angiosperm are not surrounded by cell walls and that their fusion is due to the fact that their development has stopped and that they are in a single cell. This explanation may apply to the eight fusing nuclei in *P. Sintensis*.

A peculiar phenomenon was noted in fertilization. The male and female nuclei at this time are in the resting stage and have one or more cavities with their concave sides facing each other (*fig. 29*). The edges fuse so that a mass of cytoplasm is apparently held in the fusion nucleus (*fig. 30*). The wall around this mass of cytoplasm grows faint and finally disappears.

The mature seed resembles that of *P. pellucida* (JOHNSON '00). There is a small oval embryo, while the rest of the sac is filled with a much larger endosperm, which is cellular from the first division. The sac is about the same size as at fertilization, but the cells of the nucellus have become filled with starch to form perisperm.

Peperomia arifolia

The development of *P. arifolia* was followed only as far as the sixteen-nucleate sac.

The development of the flower and of the embryo sac agrees very

closely with that just described for *P. Sintensii*. There is a single archesporial cell which cuts off a tapetal cell and then forms the embryo sac. The tapetum divides as in *P. Sintensii*. The embryo sac nucleus divides to two, four, eight, and finally sixteen nuclei.

The first division is heterotypic and shows the usual synapsis. At this division a cell wall is formed across the sac. This wall is variable in position and generally disappears before the next division, but may persist as a remnant after it. At the second division plates are formed on both spindles. One soon disappears, while the other forms a wall separating one nucleus from the other three. This wall is variable in position, and all signs of it are usually lost before the next division. The next two divisions leave the mature sac with sixteen nuclei.

Peperomia ottoniana

The material of this species was collected in Mexico by Dr. C. J. CHAMBERLAIN. It is very much like *P. Sintensii*, but shows some constant differences. It was identified by M. CASIMIR DE CANDOLLE.

Owing to lack of young material the investigation of this species had to be begun with the four-nucleate stage. There were only three ovules showing a four-nucleate sac, but they are worth recording, as they appear perfectly normal, and seem to throw some light on the problem under discussion.

The youngest sac (fig. 31) shows the micropylar nucleus completely cut off from the other three by a very distinct and well-developed wall. This nucleus is much larger than the other three, which are all about the same size. The cell occupied by this large nucleus contains much denser protoplasm than the one containing the other three. The stage just described resembles rather closely the four megasporangia of the ordinary angiosperm, where one megasporangium is to form the sac while the other three degenerate. The further development, however, seems to be different, and agrees with the two species of *Peperomia* just described.

The two other four-nucleate sacs which were seen are larger and apparently older than the one just described, and show no sign of a wall separating the nuclei, which are all about the same size (fig. 32). The eight nuclei formed from these four show no appreciable difference in size.

The further development resembles very closely that of *P. Sintensii*. The mature sac contains sixteen nuclei. There is one egg and one nucleus with the position of a synergid. Six peripheral nuclei are cut off against the wall of the sac. The remaining eight fuse to form the endosperm nucleus about the time that fertilization takes place. In the fusion of male and female nuclei there was seen no sign of protoplasm being taken into the nucleus, as described for *P. Sintensii*. Many of the stages of this species resemble *P. Sintensii* so closely that one might readily be mistaken for the other.

Peperomia pellucida

The development of the embryo sac nucleus and the structure of the mature sac, as described by JOHNSON, agree with the description just given for *P. Sintensii*. Therefore only the first two divisions of the embryo sac nucleus need be considered here.

The first division is heterotypic and shows about ten or twelve chromosomes. A meshwork contracts and goes into synapsis. This stage is followed by an apparently continuous spirem, which divides into twisted heterotypic chromosomes.

In both divisions plates are formed on the spindles. These plates probably do not develop further, as no larger plates or walls were seen, although a great number of nuclei were examined at stages which should show them if present. These plates probably are the remnants of walls such as have been described for the other three species.

Discussion

As has been said, CAMPBELL ('01) considers the unusual structure of the embryo sac of Peperomia as primitive, and expresses the belief "that the contents of the embryo sac with the sixteen nuclei represent a prothallial tissue and the nuclei are at first entirely similar." JOHNSON ('02), after a study of Peperomia and allied genera, comes to the conclusion that the peculiarities of the embryo sac have been secondarily acquired from the ordinary angiosperm embryo sac.

The present investigation seems to support the latter view. The nuclei of the mature sac of *P. Sintensii*, instead of being similar at first, bear a definite relation to each other. The presence of the reducing division in the primary embryo sac nucleus and the forma-

tion of evanescent walls in the first and second divisions seem to indicate that the sac is composed of the descendants of the nuclei of four megasporangia and that the primary embryo sac nucleus is a mother cell and not a megaspore nucleus.

If the walls corresponded to those of prothallial cells, we should expect to find them in the third division, but here not even a cell plate was seen. Besides this, the nearest phylogenetic relatives in which the first divisions of a megaspore result in a cellular structure are found among the leptosporangiate Filicales, where the heterospory is supposed to be of rather late origin, and it does not seem probable that Peperomia has reverted to the characters of an ancestor as remote as one in which we would find the first divisions of the megaspore giving rise to a cellular structure.

This position is strengthened when we consider the four-nucleate stage of the Mexican species. Here the nucleus which is cut off is considerably larger and surrounded by much denser protoplasm than the other three. The resemblance to the four megasporangia of the ordinary angiosperm is quite striking.

The presence of the extra nuclei in the mature sac is in harmony with the view that these nuclei are the descendants of four megaspore nuclei.

The nuclei of the four-nucleate sac of Peperomia have the same position with reference to each other as the nuclei of a tetrad of fern spores or of a tetrad of microspores of a spermatophyte. This position is not always apparent when the nuclei are dividing, and as the sac is somewhat rounded it may be that this arrangement is a mechanical response to the physiological conditions.

That four potential megaspore nuclei may be included in a single cell has been shown by CANNON ('00) for *Avena fatua*. Here the four megaspore nuclei may or may not be separated by cell walls, but in either case three degenerate and the other forms the embryo sac. A similar phenomenon is reported by SMITH ('98) for *Eichhornia*.

The case of *Crucianella* (LLOYD '02) is interesting in this connection. Here the four megasporangia are not separated by walls, but indications of plates are found on the spindles in the divisions of the megaspore mother cell nucleus. According to LLOYD, the four megasporangia are physiologically and morphologically similar. Each

nucleus divides to two, but afterward the nuclei derived from the three megasporangia nearest the chalazal end degenerate, while those from the one nearest the micropyle form the sac. The condition in *Crucianella* approaches that in *Peperomia*, and it may be that the shape of the sac, which is much less elongated in *Peperomia* than in *Crucianella*, gives the megasporangia a better chance to develop together in *Peperomia*.

That physiological conditions do play a part in the structure of the embryo sac of *Peperomia* may be indicated by the fact that while the megasporangium nucleus which is cut off by a cell wall is generally at the chalazal end, it is always the one nearest the micropyle which forms the functional egg apparatus.

WIEGAND ('00) reports an evanescent wall in the first division of the embryo sac of *Convallaria*. Here the mother cell forms the embryo sac directly and this wall may represent a megasporangium wall.

That more than one megasporangium may possess the potentialities for development is indicated by the number of plants in which more than one has been reported as dividing (COULTER and CHAMBERLAIN '03).

The similarity in the fate of the four megasporangia of *P. Sintensii* is striking. Each gives rise to two nuclei of the endosperm and two cut off against the embryo sac wall.

If we were to accept the view of PORSCH ('07) that an egg apparatus represents an archegonium, we might conceive of the embryo sac of *Peperomia* as really composed of four sacs, each of which gives rise to a single archegonium. The relationship of the nuclei of each egg apparatus, as previously described, is the same as that found in the egg apparatus of an ordinary angiosperm, if we assume that one synergid fuses with the nucleus which usually fuses to form the endosperm, that is, with the sister of the egg, as is the case in the four-nucleate sac of *Cypripedium* (PACE '08). That there may be in *Peperomia* four potential egg apparatuses is indicated by the fact that the nucleus of the four-nucleate stage, which is cut off and therefore resembles the functional megasporangium of the ordinary angiosperm, does not usually form the functional egg apparatus. It must be remembered, however, that no such similarity in the fate of the megasporangium nuclei as has been described for *P. Sintensii* exists in *P. his-*

pidula (JOHNSON '07), where fourteen out of sixteen nuclei fuse to form the enaosperm nucleus.

Before applying the theory of PORSCH it may be well to consider the theory itself in its relation to the ordinary gymnosperms and angiosperms. In Selaginella, Isoetes, and the gymnosperms, all of the first divisions of the megasporangium are non-cellular, after which there is a number of cellular divisions in all species that form archegonia. It is in the cellular tissue that the archegonia are formed by cell divisions. The first two divisions of the ordinary angiosperm embryo sac are generally homologized with the free nuclear divisions of the gymnosperm prothallus, and the fact that in the derived sac of *P. Sintersii*, where cell walls are formed at the first two or megasporangium-forming divisions, there is no sign of even a cell plate at the third or prothallial division, indicates that the character of the free cell divisions in the angiosperms is quite constant. It does not seem probable that nuclei formed in the angiosperm embryo sac by free nuclear division can be homologous with the nuclei in the cellular archegonia of the gymnosperms, for we would have to explain how the archegonia became shifted back from the cellular to the non-cellular phase of the prothallus.

If the above idea is correct, the polar groups in the angiosperm embryo sac can have no such phylogenetic significance as is ascribed to them by PORSCH, but all of the nuclei in the mature sac must be homologous or at least differentiated only in the last division.

From what has been said, it seems possible that in some angiosperms besides Peperomia, in which the embryo sac is not developed from one of a row of megasporangia, the first four nuclei of the sac are megasporangium nuclei. It must be remembered, however, that in most cases we have no evidence for this, other than the presence of the reducing division in the embryo sac mother cell, and it is a mistake to suppose that the same structure may not come about in plants in more than one way. Besides this, it is hard to see how four embryo sacs can have become merged into one in the large number of cases in which a row of megasporangia is not formed (COULTER and CHAMBERLAIN '03) without disturbing the normal number and position of the nuclei, as has been done in Peperomia.

Some workers have been inclined to regard the presence of the

heterotypic division in a cell of the nucellus as the sole criterion for determining that this cell is a megasporangium mother cell, and that the first four resulting nuclei are the nuclei of megaspores. This view seems to leave out of consideration the great number of points in the life-history of plants at which reduction may take place, and the evident tendency among vascular plants toward the reduction of sporogenous tissue in the megasporangium and nucellus.

In the Archegoniatae the archesporial cell may give rise to a large mass of tapetum and a considerable number of functional spore mother cells. Since we can trace the reduction of these divisions until among angiosperms the archesporial cell may without dividing form one megasporangium mother cell, it does not seem reasonable to suppose that the divisions of the mother cell to four megaspores may not also be left out and the mother cell function directly as a megasporangium. In this case the heterotypic division might be pushed forward and take place in the embryo sac.

Among the lower plants this reducing division may take place at almost any point in the life-history and there seems to be no sufficient reason for thinking that it must occur at the same place in all angiosperms.

If the two divisions which form the spores from the mother cell, or one of them, have been left out, we could of course expect to find no evidence of it other than the entire absence of any signs of the division.

In a recent paper on *Cypripedium*, Miss PACE ('08) shows that the sporogenous cell divides once and one of the resulting cells forms the embryo sac, while the other may occasionally divide once. There is not even a sign of a cell plate in the first division of the nucleus which forms the embryo sac. Miss PACE calls the first two nuclei of the embryo sac megaspores, but does not state her reasons for doing so. The question might arise as to whether they are megaspores or whether one division in spore formation has been left out. That the nucleus of the degenerating cell should occasionally divide does not seem surprising when we remember the large number of plants in which the nucleus of a degenerating megasporangium may do so.

The writer does not wish to be understood as denying that there are two megaspores in the embryo sac of *Cypripedium*, or that the

first four nuclei in the embryo sac of any plant in which a row of megasporites is not formed are megaspore nuclei, but simply as suggesting that in most cases we have no adequate proof of this, and that in the present state of our knowledge there is at least one other way in which some of them may be regarded.

The homologies in all these cases may be cleared up by further work. We have, however, no right to push them further than the present evidence justifies, or to suppose that all plants must behave alike in this respect, when we consider the large number of different analogous organs which have been arrived at by very diverse methods.

Summary

The archesporium of the species of Peperomia studied arises as a single hypodermal cell, which cuts off a single parietal cell and then forms the embryo sac directly.

The first division of the embryo sac nucleus is heterotypic. The nucleus goes into synapsis. This stage is followed by an apparently continuous spirem. This splits longitudinally but later the two halves come together again. The chromosomes are formed from loops in the spirem. When these divide, they seem to separate two parts of the spirem which were originally placed end to end.

The second division may divide the chromosomes along the longitudinal split seen in the prophase of the first division.

In the third division of the embryo sac nucleus of *P. Sintensii*, no cell plates were seen on the spindles, but in the last division cell walls are formed on all the spindles. These walls cut off, against the embryo sac walls, one of each of the eight pairs of nuclei, and leave the other eight free in the cytoplasm. These free nuclei fuse to form the endosperm nucleus. The egg and a nucleus with the position of a synergid are cousins. The other six nuclei which are cut off against the embryo sac wall finally degenerate.

The mature sac contains sixteen-nuclei, which are apparently derived from four megasporites. That the first four nuclei of the embryo sac are megaspore nuclei is indicated by the fact that the first division of the embryo sac mother cell nucleus is heterotypic and reducing, and that in *P. pellucida* cell plates are formed on the spindles of the first two divisions, while in *P. Sintensii* and *P.*

arifolia these plates grow into evanescent walls which extend across the embryo sac and separate the nuclei.

We are not justified, however, in extending the conception of four megasporangia in an embryo sac to all angiosperms in which a row of megasporangia is not formed, because we do not know that the division of the mother cell to megasporangia may not be omitted and the place of the heterotypic division be changed.

In the fertilization of *P. Sintensis* some cytoplasm appears to be taken into the nucleus.

NOTE.—Since the above was written, there have appeared two papers dealing with sixteen-nucleate embryo sacs. One is by COULTER,¹ in which he expands a suggestion offered by LLOYD in 1902, that when four megasporangia are not formed the first four nuclei of the sac are spore nuclei so far as development is concerned, and says that the formation of megasporangial nuclei cannot be omitted.

In a paper on the phylogeny of the angiosperm embryo sac, ERNST² describes a sixteen-nucleate embryo sac in Gunnera. Here he finds an egg, two synergids, six antipodal cells often in two groups of three, and seven nuclei which fuse to form the endosperm nucleus. He then attempts to fit the archegonial theory of PORSCH to Gunnera, and concludes that the egg group represents an archegonium, while two more are represented by the six antipodal cells together with two of the nuclei which fuse to form the endosperm. He thinks that the other four nuclei fail to form an archegonium, and that the explanation which he gives of the embryo sac of Gunnera may apply to *Peperomia pellucida*. In the case of the embryo sac of *P. Sintensis*, which resembles very closely that of *P. pellucida*, it has already been shown that if we should apply the theory of PORSCH there would be four and not three archegonia. ERNST has not worked out the relation between the nuclei in the embryo sac of Gunnera, and therefore it would seem premature to speculate as to the conditions there, but there seems to me to be no sufficient reason for thinking that the nuclei would represent three rather than four of the archegonia of PORSCH.

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EXPLANATION OF PLATES XXXI-XXXIII

All drawings were drawn with the aid of a camera lucida and reduced one-half. *Figs. 1, 2, 4, 18-23, 31, 32* were made with a no. 8 ocular and a 1.5^{mm} objective; *figs. 3, 5-17* with a no. 12 ocular and a 1.5^{mm} objective; *figs. 24-27, 29, 30* with a no. 8 ocular and a $\frac{1}{2}$ objective; *fig. 28* with a no. 2 ocular and a $\frac{1}{2}$ objective.

Peperomia Sintensis

FIG. 1.—Primary archesporial cell in apex of nucellus.

FIG. 2.—Archesporial cell has divided to sporogenous and parietal cell; the latter dividing and showing sixteen chromosomes; integument beginning to grow up around nucellus.

FIG. 3.—Longitudinal view of an anaphase in a vegetative cell.

FIG. 4.—Nucellus containing embryo sac mother cell, which shows a stage shortly before synapsis; part of integument which now surrounds nucellus shown at base of nucellus.

- FIG. 5.—Early stage of synapsis in embryo sac mother cell.
- FIG. 6.—Later stage of same.
- FIG. 7.—Part of an apparently continuous spirem, just after synapsis.
- FIG. 8.—Later stage of same, showing divided granules.
- FIG. 9.—Still later stage, showing split spirem.
- FIG. 10.—Still later stage; the two halves have come together and the spirem has become looped.
- FIG. 11.—Chromosomes derived from loops shown in fig. 10.
- FIG. 12.—Later stage of same.
- FIG. 13.—Still later stage.
- FIG. 14.—Still later stage; the two halves have come together.
- FIG. 15.—Longitudinal section of a metaphase of the first division of embryo sac mother cell.
- FIG. 16.—Anaphase of same.
- FIG. 17.—Early stage in formation of daughter nuclei.
- FIG. 18.—Two-nucleate embryo sac, showing cross-wall separating the two nuclei.
- FIG. 19.—Slightly older embryo sac.
- FIG. 20.—Embryo sac showing same stage as last.
- FIG. 21.—Nuclei of a two-nucleate sac dividing; remnant of dividing wall still present.
- FIG. 22.—Four-nucleate sac; one nucleus separated from the other three by a wall.
- FIG. 23.—Later stage; wall was disappeared.
- FIG. 24.—Four nuclei have divided to eight; no cell plates on the spindles; the sister nuclei in micropylar end.
- FIG. 25.—Later stage; the two sister nuclei still in micropylar end.
- FIG. 26.—The eight nuclei have just divided to sixteen; cell plates seen on the spindles.
- FIG. 27.—Later stage; cell plates shown in last figure have grown into walls cutting off eight nuclei against the embryo sac wall and leaving eight free in the cytoplasm.
- FIG. 28.—A mature ovule; the embryo sac contains sixteen nuclei; *p*, pollen tube with nuclei; *t*, tapetum; *i*, integument.
- FIG. 29.—Embryo sac with fertilized egg and nuclei fusing to form the endosperm nucleus; *e*, egg with fusing nuclei; *p*, pollen tube; *s*, nucleus with the position of a synergid; *d*, peripheral nuclei which will degenerate; *f*, nuclei fusing to form endosperm nucleus.
- FIG. 30.—Later stage of fusion in male and female nuclei.

Peperomia ottoniana

- FIG. 31.—Four-nucleate sac; one nucleus cut off and surrounded by dense protoplasm.
- FIG. 32.—Later stage; the wall dividing off one nucleus has disappeared.





